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FORM PTO-1390 OFFICE		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER <b>PF-0698 USN</b>
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 CFR 1.5) TO BE ASSIGNED <b>10/019495</b>
INTERNATIONAL APPLICATION NO. <b>PCT/US00/10884</b>	INTERNATIONAL FILING DATE <b>20 April 2000</b>	PRIORITY DATE CLAIMED <b>23 April 1999</b>	
TITLE OF INVENTION <b>HUMAN MEMBRANE-ASSOCIATED PROTEINS</b>			
APPLICANT(S) FOR DO/EO/US <b>HILLMAN, Jennifer L.; BANDMAN, Olga; TANG, Y. Tom; LAL, Preeti; YUE, Henry; REDDY, Roopa; AZIMZAI, Yalda; BAUGHN, Mariah R.</b>			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: 1. <input checked="" type="checkbox"/> This is the <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to promptly begin national examination procedures (35 U.S.C. 371 (f)). 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (PCT Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau) b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).			
<b>Items 11 to 16 below concern document(s) or information included:</b> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.27 and 3.31 is included. 13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment, as follows: Cancel in this application original claims #16, 19, and 22 before calculating the filing fee, without prejudice or disclaimer. Applicants submit that these claims were included in the application as filed in the interest of providing notice to the public of certain specific subject matter intended to be claimed, and are being canceled at this time in the interest of reducing filing costs. Applicants expressly state that these claims are not being canceled for reasons related to patentability, and are in fact fully supported by the specification as filed. Applicants expressly reserve the right to reinstate these claims or to add other claims during prosecution of this application or a continuation or divisional application. Applicants expressly do not disclaim the subject matter of any invention disclosed herein which is not set forth in the instantly filed claims. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: 1) Transmittal Letter (2 pp, in duplicate) 2) Return Postcard 3) Express Mail Label No.: <b>EL 856 148772 US</b> 4) Request to Transfer 5) Assignment (2 pp) and Assignment Cover Sheet (1page) 6) Oath & Declaration (5 pp)			

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) <b>TO BE ASSIGNED 10/019495</b>	INTERNATIONAL APPLICATION NO.: PCT/US00/10882	ATTORNEY'S DOCKET NUMBER PF-0698 USN
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17. ☒ The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....\$1000.00

☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..\$860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$710.00

☒ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....\$690.00

☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....\$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	\$740.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	\$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	20 =	0	X \$ 18.00	\$	
Independent Claims	2 =	0	X \$ 80.00	\$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$	
<b>SUBTOTAL</b>				\$740.00	
=					
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1492(f)).				\$	
<b>TOTAL NATIONAL FEE =</b>				\$740.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by the appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$0.00	
<b>TOTAL FEES ENCLOSED =</b>				\$740.00	

	Amount to be Refunded:	\$
	Charged:	\$

a. ☐ A check in the amount of \$ \_\_\_\_\_ to cover the above fees is enclosed.

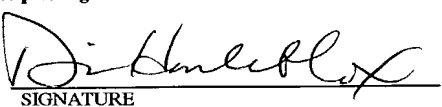
b. ☒ Please charge my Deposit Account No. 09-0108, in the amount of \$740.00 to cover the above fees.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 09-0108. A duplicate copy of this sheet is enclosed.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

INCYTE GENOMICS, INC.  
 3160 Porter Drive  
 Palo Alto, CA 94304

  
 SIGNATURE

NAME: Diana Hamlet-Cox

REGISTRATION NUMBER: 33,302

DATE: 23 October 2001

## HUMAN MEMBRANE-ASSOCIATED PROTEINS

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human membrane-associated proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cell signaling, cell differentiation, and cell proliferation disorders.

### BACKGROUND OF THE INVENTION

Eukaryotic cells are surrounded by plasma membranes which enclose the cell and maintain an environment inside the cell that is distinct from its surroundings. In addition, eukaryotic organisms are distinct from prokaryotes in that they possess many intracellular organelle and vesicle structures. Many of the metabolic reactions which distinguish eukaryotic biochemistry from prokaryotic biochemistry take place within these structures. The plasma membrane and the membranes surrounding organelles and vesicles are composed of phosphoglycerides, fatty acids, cholesterol, phospholipids, glycolipids, proteoglycans, and proteins. These components confer identity and functionality to the membranes with which they associate.

#### Integral Membrane Proteins

The majority of known integral membrane proteins are transmembrane proteins (TM) which are characterized by an extracellular, a transmembrane, and an intracellular domain. TM domains are typically comprised of 15 to 25 hydrophobic amino acids which are predicted to adopt an  $\alpha$ -helical conformation. TM proteins are classified as bitopic (Types I and II) and polytopic (Types III and IV) (Singer, S.J. (1990) Annu. Rev. Cell Biol. 6:247-96). Bitopic proteins span the membrane once while polytopic proteins contain multiple membrane-spanning segments. TM proteins function as cell-surface receptors, receptor-interacting proteins, transporters of ions or metabolites, ion channels, cell anchoring proteins, and cell type-specific surface antigens.

Many membrane proteins (MPs) contain amino acid sequence motifs that target these proteins to specific subcellular sites. Examples of these motifs include PDZ domains, KDEL, RGD, NGR, and GSL sequence motifs, von Willebrand factor A (vWFA) domains, and EGF-like domains. RGD, NGR, and GSL motif-containing peptides have been used as drug delivery agents in cancer treatments which target tumor vasculature (Arap, W. et al. (1998) Science, 279:377-380). Furthermore, MPs may also contain amino acid sequence motifs, such as the carbohydrate recognition domain (CRD), that mediate interactions with extracellular or intracellular molecules.

In some cases TM proteins function as mediators of cell-cell attachment. For example, Emp (erythrocyte macrophage protein), which mediates attachment of erythroblasts to macrophages, has a putative TM domain near its N-terminus. It is postulated that Emp suppresses the process of

apoptosis by promoting terminal differentiation of erythroid cells when Emp-mediated contact is made between erythroblasts and macrophages (Hanspal, M. et al. (1998) Blood 92:2940-2950).

One function of TM proteins is to facilitate cell-cell communication. Neurexins are a family of neuronal cell surface receptor proteins, with single TM regions, that aid in axon guidance and synaptogenesis. Neurexins exhibit extensive alternative splicing that produces hundreds of unique  
5 neurexins in the brain (Ushkaryov, Y.A. et. al. (1992) Science 257:50-56).

In some cases TM proteins serve as transporters or channels in the cell membrane. The Rh (Rhesus) erythrocyte blood group protein family serve such a function in erythrocyte membranes. The family includes both Rh50 glycoprotein and Rh30 polypeptides that together form a complex  
10 essential for Rh antigen expression and erythrocyte membrane integrity. Mutations in Rh50 underlie Rh deficiency syndrome, an autosomal recessive disorder associated with chronic hemolytic anemia and spheromatocytosis (Huang, C.H. (1998) J. Biol. Chem. 273:2207-2213).

Tumor antigens are cell surface molecules that are differentially expressed in tumor cells relative to normal cells. Tumor antigens distinguish tumor cells immunologically from normal cells  
15 and provide diagnostic and therapeutic targets for human cancers. (Takagi, S. et al. (1995) Int. J. Cancer 61: 706-715; Liu, E. et al. (1992) Oncogene 7: 1027-1032.)

Other types of cell surface antigens include those identified on leukocytic cells of the immune system. These antigens have been identified using systematic, monoclonal antibody (mAb)-based "shot gun" techniques. These techniques have resulted in the production of hundreds of mAbs  
20 directed against unknown cell surface leukocytic antigens. These antigens have been grouped into "clusters of differentiation" based on common immunocytochemical localization patterns in various differentiated and undifferentiated leukocytic cell types. Antigens in a given cluster are presumed to identify a single cell surface protein and are assigned a "CD" or "cluster of differentiation" designation. Some of the genes encoding proteins identified by CD antigens have been cloned and  
25 verified by standard molecular biology techniques. CD antigens have been characterized as both transmembrane proteins and cell surface proteins anchored to the plasma membrane via covalent attachment to fatty acid-containing glycolipids such as glycosylphosphatidylinositol (GPI), discussed below. (Reviewed in Barclay, A. N. et al. (1995) The Leucocyte Antigen Facts Book, Academic Press, San Diego, CA, pp. 17-20.)

30 The TM cell surface glycoprotein CD69 is an early activation antigen of T lymphocytes. CD69 is homologous to members of a supergene family of type II integral membrane proteins having C-type lectin domains. Although the precise functions of the CD-69 antigen is not known, evidence suggests that these proteins transmit mitogenic signals across the plasma membrane and are up-regulated in response to lymphocyte activation (Hammann, J. et. al. (1993) J. Immunol. 150:4920-  
35 4927).



sequence selected from the group consisting of SEQ ID NO:1-17. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-17.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17. In one alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:18-34.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17.



and b) detecting agonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional HUMAP,  
5 comprising administering to a patient in need of such treatment the pharmaceutical composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, c) a  
10 biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an antagonist compound identified by the method  
15 and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional HUMAP, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a  
20 sequence selected from the group consisting of SEQ ID NO:18-34, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

# **BRIEF DESCRIPTION OF THE TABLES**

25 Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HUMAP.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of HUMAP.

30 Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding HUMAP were isolated.

35 Table 5 shows the tools, programs, and algorithms used to analyze HUMAP, along with



applicable descriptions, references, and threshold parameters.

### DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood  
5 that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an,"  
10 and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings  
15 as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in  
20 connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

### DEFINITIONS

"HUMAP" refers to the amino acid sequences of substantially purified HUMAP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and  
25 human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of HUMAP. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HUMAP either by directly interacting with HUMAP or by acting on components of the biological pathway in which HUMAP participates.

30 An "allelic variant" is an alternative form of the gene encoding HUMAP. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides.

Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

“Altered” nucleic acid sequences encoding HUMAP include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as HUMAP or a polypeptide with at least one functional characteristic of HUMAP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HUMAP, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HUMAP. The encoded protein may also be “altered,” and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HUMAP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HUMAP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms “amino acid” and “amino acid sequence” refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where “amino acid sequence” is recited to refer to an amino acid sequence of a naturally occurring protein molecule, “amino acid sequence” and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

“Amplification” relates to the production of additional copies of a nucleic acid sequence.

Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term “antagonist” refers to a molecule which inhibits or attenuates the biological activity of HUMAP. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HUMAP either by directly interacting with HUMAP or by acting on components of the biological pathway in which HUMAP participates.

The term “antibody” refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind HUMAP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or

oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize  
5 the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the  
10 protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates,  
15 methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence  
20 produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability  
25 of the natural, recombinant, or synthetic HUMAP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial,"  
30 such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acid strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution.

Compositions comprising polynucleotide sequences encoding HUMAP or fragments of HUMAP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of one or more Incyte Clones and, in some cases, one or more public domain ESTs, using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser
Gln	Asn, Glu, His
Glu	Asp, Gln, His
Gly	Ala
His	Asn, Arg, Gln, Glu
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	His, Met, Leu, Trp, Tyr
Ser	Cys, Thr
Thr	Ser, Val
Trp	Phe, Tyr
Tyr	His, Phe, Trp
Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide

backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation.

(b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the  
5 absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of  
10 the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "fragment" is a unique portion of HUMAP or the polynucleotide encoding HUMAP which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise  
15 up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a  
20 molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:18-34 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:18-34, for example, as distinct from any other sequence in the same genome. A fragment of SEQ ID NO:18-34 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:18-34 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:18-34 and the region of  
30 SEQ ID NO:18-34 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-17 is encoded by a fragment of SEQ ID NO:18-34. A fragment of SEQ ID NO:1-17 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-17. For example, a fragment of SEQ ID NO:1-17 is useful as an immunogenic peptide  
35 for the development of antibodies that specifically recognize SEQ ID NO:1-17. The precise length of



<http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*  
*Reward for match: 1*  
*Penalty for mismatch: -2*  
*Open Gap: 5 and Extension Gap: 2 penalties*  
*Gap x drop-off: 50*  
*Expect: 10*  
*Word Size: 11*  
*Filter: on*

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and  
 5 "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9  
 10 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Open Gap: 11 and Extension Gap: 1 penalties*

*Gap x drop-off: 50*

*Expect: 10*

15 *Word Size: 3*

*Filter: on*

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance,  
 20 a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain  
 25 DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

30 "Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the



hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating  $T_m$  and conditions for nucleic acid hybridization are well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

5 An "immunogenic fragment" is a polypeptide or oligopeptide fragment of HUMAP which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of HUMAP which is useful in any of the antibody production methods disclosed herein or known in the art.

10 The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate. The terms "element" and "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate" refers to a change in the activity of HUMAP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, 15 functional, or immunological properties of HUMAP.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

20 "Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

25 "Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

30 "Probe" refers to nucleic acid sequences encoding HUMAP, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by 35 complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA

polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al., 1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing

primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, *supra*. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding HUMAP, or fragments thereof, or HUMAP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by

different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides

due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule.

Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

## THE INVENTION

The invention is based on the discovery of new human membrane-associated proteins (HUMAP), the polynucleotides encoding HUMAP, and the use of these compositions for the diagnosis, treatment, or prevention of cell signaling, cell differentiation, and cell proliferation disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding HUMAP. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each HUMAP were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries.

The Incyte clones in column 5 were used to assemble the consensus nucleotide sequence of each HUMAP and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding HUMAP. The first column of Table 3 lists the

nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:18-34 and to distinguish between SEQ ID NO:18-34 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express HUMAP as a fraction of total tissues expressing HUMAP. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing HUMAP as a fraction of total tissues expressing HUMAP. Column 5 lists the vectors used to subclone each cDNA library.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding HUMAP were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO:31 maps to chromosome 4 within the interval from 77.30 to 88.50 centiMorgans.

The invention also encompasses HUMAP variants. A preferred HUMAP variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the HUMAP amino acid sequence, and which contains at least one functional or structural characteristic of HUMAP.

The invention also encompasses polynucleotides which encode HUMAP. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:18-34, which encodes HUMAP. The polynucleotide sequences of SEQ ID NO:18-34, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding HUMAP. In particular, such a variant polynucleotide sequence will have at least about 80%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HUMAP. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:18-34 which has at least about 80%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:18-34. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HUMAP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HUMAP, some bearing minimal similarity to

the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HUMAP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HUMAP and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring HUMAP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HUMAP or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HUMAP and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HUMAP and HUMAP derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HUMAP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:18-34 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Perkin-Elmer).



Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular  
 5 Biology. John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding HUMAP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed,

10 restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.)

Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids

15 Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences  
 20 are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060).

Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences,  
 25 Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include  
 30 sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary

sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process  
 5 from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HUMAP may be cloned in recombinant DNA molecules that direct expression of HUMAP, or  
 10 fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HUMAP.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HUMAP-encoding sequences for a variety of purposes including, but  
 15 not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Cramer, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or  
 20 improve the biological properties of HUMAP, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial"  
 25 breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable  
 30 manner.

In another embodiment, sequences encoding HUMAP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) *Nucleic Acids Symp. Ser.* 7:215-223; and Horn, T. et al. (1980) *Nucleic Acids Symp. Ser.* 7:225-232.) Alternatively, HUMAP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide  
5 synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) *Science* 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HUMAP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

10 The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

15 In order to express a biologically active HUMAP, the nucleotide sequences encoding HUMAP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in  
20 polynucleotide sequences encoding HUMAP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HUMAP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HUMAP and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or  
25 translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g.,  
30 Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HUMAP and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A

Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HUMAP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HUMAP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HUMAP can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HUMAP into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HUMAP are needed, e.g. for the production of antibodies, vectors which direct high level expression of HUMAP may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HUMAP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HUMAP. Transcription of sequences encoding HUMAP may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al.

(1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

5 In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HUMAP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HUMAP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

15 Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

20 For long term production of recombinant proteins in mammalian systems, stable expression of HUMAP in cell lines is preferred. For example, sequences encoding HUMAP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

25 Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*<sup>-</sup> and *apr*<sup>-</sup> cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements

for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP: Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of  
5 transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HUMAP is inserted within a marker gene sequence, transformed cells containing  
10 sequences encoding HUMAP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HUMAP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HUMAP and that express  
15 HUMAP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HUMAP using either  
20 specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HUMAP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et  
25 al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization  
30 or PCR probes for detecting sequences related to polynucleotides encoding HUMAP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HUMAP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase

such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HUMAP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HUMAP may be designed to contain signal sequences which direct secretion of HUMAP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HUMAP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HUMAP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HUMAP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HUMAP encoding sequence and the heterologous protein sequence, so that

HUMAP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, *supra*, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HUMAP may be achieved *in vitro* using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example,  $^{35}\text{S}$ -methionine.

Fragments of HUMAP may be produced not only by recombinant means, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, *supra*, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of HUMAP may be synthesized separately and then combined to produce the full length molecule.

#### THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HUMAP and human membrane-associated proteins. In addition, the expression of HUMAP is closely associated with cell proliferation, cancer, and inflammation. Therefore, HUMAP appears to play a role in cell signaling, cell differentiation, and cell proliferation disorders. In the treatment of disorders associated with increased HUMAP expression or activity, it is desirable to decrease the expression or activity of HUMAP. In the treatment of disorders associated with decreased HUMAP expression or activity, it is desirable to increase the expression or activity of HUMAP.

Therefore, in one embodiment, HUMAP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HUMAP. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; a cell differentiation disorder including developmental disorders such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation),



Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss and disorders of immune cell activation; and a disorder of cell signaling including endocrine disorders such as disorders of the hypothalamus and pituitary resulting from lesions such as primary brain tumors, adenomas, infarction associated with pregnancy, hypophysectomy, aneurysms, vascular malformations, thrombosis, infections, immunological disorders, and complications due to head trauma; disorders associated with hyperpituitarism including acromegaly, gigantism, and syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) often caused by benign adenoma; disorders associated with hypothyroidism including goiter, myxedema, acute thyroiditis associated with bacterial infection; disorders associated with hyperparathyroidism including Conn disease (chronic hypercalcemia); pancreatic disorders such as Type I or Type II diabetes mellitus and associated complications; disorders associated with the adrenals such as hyperplasia, carcinoma, or adenoma of the adrenal cortex, hypertension associated with alkalosis; disorders associated with gonadal steroid hormones such as: in women, abnormal prolactin production, infertility, endometriosis, perturbations of the menstrual cycle, polycystic ovarian disease, hyperprolactinemia, isolated gonadotropin deficiency, amenorrhea, galactorrhea, hermaphroditism, hirsutism and virilization, breast cancer, and, in post-menopausal women, osteoporosis; and, in men, Leydig cell deficiency, male climacteric phase, and germinal cell aplasia, hypergonadal disorders associated with Leydig cell tumors, androgen resistance associated with absence of androgen receptors, syndrome of  $\alpha$ -reductase, and gynecomastia.

In another embodiment, a vector capable of expressing HUMAP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HUMAP including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HUMAP in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HUMAP including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of HUMAP may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HUMAP including, but not limited to, those listed above.

In a further embodiment, an antagonist of HUMAP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HUMAP. Examples of such disorders include, but are not limited to, those cell signaling, cell differentiation, and cell proliferation

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disorders described above. In one aspect, an antibody which specifically binds HUMAP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HUMAP.

In an additional embodiment, a vector expressing the complement of the polynucleotide  
5 encoding HUMAP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HUMAP including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by  
10 one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HUMAP may be produced using methods which are generally known in the  
15 art. In particular, purified HUMAP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HUMAP. Antibodies to HUMAP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit  
20 dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HUMAP or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels  
25 such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HUMAP have an amino acid sequence consisting of at least about 5 amino acids, and generally will  
30 consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HUMAP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HUMAP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J.*

5 Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:2026-2030; and Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) *Proc.*

10 Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda, S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HUMAP-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton,  
15 D.R. (1991) *Proc. Natl. Acad. Sci. USA* 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:3833-3837; Winter, G. et al. (1991) *Nature* 349:293-299.)

20 Antibody fragments which contain specific binding sites for HUMAP may also be generated. For example, such fragments include, but are not limited to, F(ab')<sub>2</sub> fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al.  
25 (1989) *Science* 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HUMAP and its  
30 specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HUMAP epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for HUMAP. Affinity is expressed as an association

constant,  $K_a$ , which is defined as the molar concentration of HUMAP-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The  $K_a$  determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HUMAP epitopes, represents the average affinity, or avidity, of the antibodies for HUMAP. The  $K_a$  determined for a preparation of monoclonal antibodies, which are monospecific for a particular HUMAP epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  L/mole are preferred for use in immunoassays in which the HUMAP-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with  $K_a$  ranging from about  $10^6$  to  $10^7$  L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of HUMAP, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington, DC; Liddell, J.E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of HUMAP-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding HUMAP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding HUMAP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HUMAP. Thus, complementary molecules or fragments may be used to modulate HUMAP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding HUMAP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HUMAP. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

Genes encoding HUMAP can be turned off by transforming a cell or tissue with expression



suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible  
 5 modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine,  
 10 guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved  
 15 using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

20 An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HUMAP, antibodies to HUMAP, and mimetics, agonists, antagonists, or inhibitors of HUMAP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound,  
 25 which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary,  
 30 intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on

techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like. for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable

stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

5 The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acids.

10 Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate  
15 container and labeled for treatment of an indicated condition. For administration of HUMAP, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

20 For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

25 A therapeutically effective dose refers to that amount of active ingredient, for example HUMAP or fragments thereof, antibodies of HUMAP, and agonists, antagonists or inhibitors of HUMAP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the  $ED_{50}$  (the dose therapeutically effective in 50% of the population) or  $LD_{50}$  (the  
30 dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the  $LD_{50}/ED_{50}$  ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the  $ED_{50}$  with little



or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1  $\mu\text{g}$  to 100,000  $\mu\text{g}$ , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

## DIAGNOSTICS

In another embodiment, antibodies which specifically bind HUMAP may be used for the diagnosis of disorders characterized by expression of HUMAP, or in assays to monitor patients being treated with HUMAP or agonists, antagonists, or inhibitors of HUMAP. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for HUMAP include methods which utilize the antibody and a label to detect HUMAP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HUMAP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HUMAP expression. Normal or standard values for HUMAP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to HUMAP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of HUMAP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HUMAP may be used for

diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of HUMAP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HUMAP, and to monitor regulation of HUMAP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HUMAP or closely related molecules may be used to identify nucleic acid sequences which encode HUMAP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding HUMAP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the HUMAP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:18-34 or from genomic sequences including promoters, enhancers, and introns of the HUMAP gene.

Means for producing specific hybridization probes for DNAs encoding HUMAP include the cloning of polynucleotide sequences encoding HUMAP or HUMAP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HUMAP may be used for the diagnosis of disorders associated with expression of HUMAP. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; a cell differentiation disorder including developmental disorders such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental

retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss and disorders of immune cell activation; and a disorder of cell signaling including endocrine disorders such as disorders of the hypothalamus and pituitary resulting from lesions such as primary brain tumors, adenomas, infarction associated with pregnancy, hypophysectomy, aneurysms, vascular malformations, thrombosis, infections, immunological disorders, and complications due to head trauma; disorders associated with hyperpituitarism including acromegaly, giantism, and syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) often caused by benign adenoma; disorders associated with hypothyroidism including goiter, myxedema, acute thyroiditis associated with bacterial infection; disorders associated with hyperparathyroidism including Conn disease (chronic hypercalcemia); pancreatic disorders such as Type I or Type II diabetes mellitus and associated complications; disorders associated with the adrenals such as hyperplasia, carcinoma, or adenoma of the adrenal cortex, hypertension associated with alkalosis; disorders associated with gonadal steroid hormones such as: in women, abnormal prolactin production, infertility, endometriosis, perturbations of the menstrual cycle, polycystic ovarian disease, hyperprolactinemia, isolated gonadotropin deficiency, amenorrhea, galactorrhea, hermaphroditism, hirsutism and virilization, breast cancer, and, in post-menopausal women, osteoporosis; and, in men, Leydig cell deficiency, male climacteric phase, and germinal cell aplasia, hypergonadal disorders associated with Leydig cell tumors, androgen resistance associated with absence of androgen receptors, syndrome of 5  $\alpha$ -reductase, and gynecomastia. The polynucleotide sequences encoding HUMAP may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HUMAP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HUMAP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HUMAP may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HUMAP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the

treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HUMAP, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HUMAP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HUMAP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding HUMAP, or a fragment of a polynucleotide complementary to the polynucleotide encoding HUMAP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

Methods which may also be used to quantify the expression of HUMAP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HUMAP may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding HUMAP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may

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represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

5 In another embodiment of the invention, HUMAP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HUMAP and the agent being tested may be measured.

10 Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HUMAP, or fragments thereof, and washed. Bound HUMAP is then detected by methods well known in the art. Purified HUMAP can  
15 also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HUMAP specifically compete with a test compound for binding  
20 HUMAP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HUMAP.

In additional embodiments, the nucleotide sequences which encode HUMAP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such  
25 properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

30 The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/130,694 and U.S. Ser. No. 60/140,580, are hereby expressly incorporated by reference.

## EXAMPLES

### I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A<sup>+</sup>) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, *supra*, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 $\alpha$ , DH10B, or ElectroMAX DH10B from Life Technologies.

### II. Isolation of cDNA Clones

Plasmids were recovered from host cells by *in vivo* excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN.

Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

### III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, *supra*, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VI.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.



The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:18-34. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

#### IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may

identify related molecules.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding HUMAP occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table

3.

#### V. Chromosomal Mapping of HUMAP Encoding Polynucleotides

The cDNA sequences which were used to assemble SEQ ID NO:27-34 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:18-34 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO:, to that map location.

The genetic map location of SEQ ID NO:31 is described in The Invention as range, or interval, of a human chromosome. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters.

#### VI. Extension of HUMAP Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:18-34 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at

temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

5 High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing  $Mg^{2+}$ ,  $(NH_4)_2SO_4$ , and  $\beta$ -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

15 The concentration of DNA in each well was determined by dispensing 100  $\mu$ l PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5  $\mu$ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For 25 shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing 30 media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2,

3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:18-34 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

## 10 VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:18-34 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10<sup>7</sup> counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

## VIII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, *supra*.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of

fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

#### IX. Complementary Polynucleotides

Sequences complementary to the HUMAP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HUMAP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HUMAP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HUMAP-encoding transcript.

#### X. Expression of HUMAP

Expression and purification of HUMAP is achieved using bacterial or virus-based expression systems. For expression of HUMAP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac (tac)* hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express HUMAP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of HUMAP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding HUMAP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong

polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, HUMAP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from HUMAP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified HUMAP obtained by these methods can be used directly in the following activity assay.

## XI. Demonstration of HUMAP Activity

HUMAP activity is demonstrated using a generic immunoblotting strategy or through a HUMAP-specific activity assay as outlined below. As a general approach, cell lines or tissues transformed with a vector containing HUMAP coding sequences can be assayed for HUMAP activity by immunoblotting. Transformed cells are denatured in SDS in the presence of  $\beta$ -mercaptoethanol, nucleic acids are removed by ethanol precipitation, and proteins are purified by acetone precipitation. Pellets are resuspended in 20 mM tris buffer at pH 7.5 and incubated with Protein G-Sepharose pre-coated with an antibody specific for HUMAP. After washing, the Sepharose beads are boiled in electrophoresis sample buffer, and the eluted proteins subjected to SDS-PAGE. Proteins are transferred from the SDS-PAGE gel to a membrane for immunoblotting, and the HUMAP activity is assessed by visualizing and quantifying bands on the blot using antibody specific for HUMAP as the primary antibody and  $^{125}\text{I}$ -labeled IgG specific for the primary antibody as the secondary antibody.

A specific assay for HUMAP activity measures the expression of HUMAP on the cell surface. cDNA encoding HUMAP is transfected into a mammalian (non-human) cell line. Cell surface proteins are labeled with biotin as described in de la Fuente, M.A.. et al. ((1997) Blood 90:2398-2405). Immunoprecipitations are performed using HUMAP-specific antibodies, and immunoprecipitated samples are analyzed using SDS-PAGE and immunoblotting techniques. The ratio of labeled

immunoprecipitant to unlabeled immunoprecipitant is proportional to the amount of HUMAP expressed on the cell surface.

## XII. Functional Assays

HUMAP function is assessed by expressing the sequences encoding HUMAP at

physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression.

Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are

transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line,

using either liposome formulations or electroporation. 1-2  $\mu\text{g}$  of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a

means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an

automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64.

GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell

death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90

degree side light scatter; down-regulation of DNA synthesis as measured by decrease in

measured by reactivity with specific antibodies; and alterations in plasma membrane composition as

measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of HUMAP on gene expression can be assessed using highly purified

populations of cells transfected with sequences encoding HUMAP and either CD64 or CD64-GFP.

CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells

using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art.

Expression of mRNA encoding HUMAP and other genes of interest can be analyzed by northern analysis or microarray techniques.

### **XIII. Production of HUMAP Specific Antibodies**

HUMAP substantially purified using polyacrylamide gel electrophoresis (PAGE: see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HUMAP amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, *supra*, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for anti-peptide and anti-HUMAP activity by, for example, binding the peptide or HUMAP to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

### **XIV. Purification of Naturally Occurring HUMAP Using Specific Antibodies**

Naturally occurring or recombinant HUMAP is substantially purified by immunoaffinity chromatography using antibodies specific for HUMAP. An immunoaffinity column is constructed by covalently coupling anti-HUMAP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HUMAP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HUMAP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HUMAP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HUMAP is collected.

### **XV. Identification of Molecules Which Interact with HUMAP**

HUMAP, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HUMAP, washed, and any wells with labeled HUMAP complex are assayed. Data obtained using different concentrations of HUMAP are used to calculate values for the number, affinity, and association of HUMAP with the candidate molecules.



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Alternatively, molecules interacting with HUMAP are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989, Nature 340:245-246), or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

5 Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious  
10 to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	18	402771	TMLR3DT01	402771H1 and 402771R6 (TMLR3DT01), 1466864F1 (PANCUTUT02), 1509777F1 (LUNGNOT14), 3114223F6 (BRSTNOT17)
2	19	1296216	PGANNOT03	1296216F6 and 1296216H1 (PGANNOT03), 3722537T6 (BRSTNOT23), 4632932F6 (GBLADIT02)
3	20	1693173	COLNNOT23	693242X11 and 693242X12 (LUNGUTUT02), 811145R1 and 811145T1 (LUNGNOT04), 1651914F6 (PROSTUT08), 1693173H1 (COLNNOT23), 1728640H1 (PROSNOT14), 1928183T6 (BRSTNOT02), 2189248X11F1 (PROSNOT26)
4	21	2095069	BRAITUT02	644829R6 (BRSTTUT02), 1805492F6 (SINTNOT13), 1967480R6 (BRSTNOT04), 2054057R6 and 2054057T6 (BEPINOT01), 2095069H1 (BRAITUT02), 4842212H1 (OSTENOT01)
5	22	2645927	OVARTUT04	2645927H1 (OVARTUT04), 3055070F6 (LNODNOT08), SBZA00651V1, SBZA00323V1, SBZA02480V1, SBZA01011V1, SBZA02764V1
6	23	2732365	OVARTUT04	1384071F1 (BRAITUT08), 2732365H1 (OVARTUT04), 2957169F6 and 2957169T6 (KIDNFET01), 3442683H1 (PENCNOT06), 5576210H1 (BRAPNOT04)
7	24	3536577	KIDNNOT25	136112H1 (SYNORAB01), 754315R1 (BRAITUT02), 2514029T6 (LIVRTUT04), 3536577H1 (KIDNNOT25)
8	25	5587790	ENDINOT02	875452R1 (LUNGAST01), 1281518T1 (COLNNOT16), 1499814F1 (SINTBEST01), 2695347H1 (UTRSNOT12), 5587790H1 (ENDINOT02), SATB00548F1, SATB00117F1
9	26	5733930	KIDCTMT01	142450F1 (TLYNNOR01), 489394R6 (HNT2AGT01), 676769R6 (CRBLNOT01), 996762R1 (KIDNTUT01), 1620177T6 (BRAITUT13), 1880279F6 (LEUKNOT03), 1959869R6 (BRSTNOT04), 2138005F6 (ENDCNOT01), 2195440F6 (THP1NOT01), 2660706F6 (LUNGUTUT09), 2866032F6 (KIDNNOT20), 3099462H1 (CERVNOT03), 5733930H1 (KIDCTMT01)
10	27	645566	BRSTTUT02	645566H1 (BRSTTUT02), 645566T6 (BRSTTUT02), 2219640F6 (LUNGNOT18), 2667331T6 (ESOGTUT02), 2791342F6 (COLNTUT16), 3689033H1 (HEAANOT01), SBBA03918F1, SBBA04464F1, SBBA05733F1
11	28	1503111	BRAITUT07	1426356H1 (SINTBEST01), 1503111H1 (BRAITUT07), 3494933F6 (ADRETUT07), 4309056F6 (BRAUNOT01), SBRA03432D1.comp, SBRA01635D1.comp, SBRA04091D1, SBRA00844D1.comp, SBRA04019D1, SBRA04382D1, SBRA03157D1

Table 1 (cont'd)

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
12	29	1984287	LUNGAST01	488790R7 (HNT2AGT01), 488790T7 (HNT2AGT01), 1390203H1 (EOSINOT01), 1984287H1 (LUNGAST01), 1984287R6 (LUNGAST01), 3636154H1 (LIVRNOT03), SATB00684F1, SAZA01496F1, SATB00480F1, SATB00505F1, SATB00419F1
13	30	2055289	BEPINOT01	936961T1 (CERVNOT01), 1452058F1 (PENITUT01), 1819965F6 (GBLATUT01), 2055289H1 (BEPINOT01), 3095524H1 (CERVNOT03), 3381923H1 (ESOGNOT04), 3814228F6 (TONSNOT03), 4554433H1 (KERAUNT01), 4556224H1 (KERAUNT01)
14	31	2279216	PROSNON01	589153X13 (UTRSNOT01), 1345342F6 (PROSNOT11), 2279216H1 (PROSNON01), 2279216T6 (PROSNON01), 2308003X12C1 (NGANNOT01), 2600037F6 (UTRSNOT10), 2600037T6 (UTRSNOT10), 3382277F6 (ESOGNOT04), SAGA00340R1, SAGA00477R1
15	32	2590650	LUNGNOT22	2590650H1 (LUNGNOT22), 2591745F6 (LUNGNOT22), 2591745T6 (LUNGNOT22)
16	33	2814726	OVARNOT10	2665352F6 (ADRENOT08), 2814726H1 (OVARNOT10), 2814726T6 (OVARNOT10), 3248008H1 (SEMVNOT03), 3249557F7 (SEMVNOT03), SBLA02275F1, SBLA01251F1
17	34	4628933	FIBRTXT02	2626682F6 (PROSTUT12), 4628933F6 (FIBRTXT02), 4628933H1 (FIBRTXT02)

Table 2

Poly-peptide Seq ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Genbank homologs	Homologous sequences	Analytical Methods
1	175	S30 S75 T81 S161	N70 N136	Transmembrane domain I88-L114; Y116-N136; G139-I158	Proteolipid protein 2 [Mus musculus] (g5771451)	HMMER BLAST
2	161	T19	N59	Transmembrane domain M1-E18; T113-L132; M131- V147	Endogenous retrovirus envelope protein (g1196425)	HMMER BLAST
3	563	S11 S55 S62 S127 T135 S139 T141 S204 S332 S338 S362 S432 T154 S169 T198 T446 S514 Y463	N3 N26 N53 N79 N97 N133 N148 N196 N203 N251 N270 N336 N343 N443	Transmembrane domain F520-V541	mucin Muc4 [Mus musculus] (g6685155)	HMMER BLAST
4	396	T175 S69 S149 T162 S194 S226 S231 S388 T13 T21 S95 S199 S308 S369	N185 N282		Erythroblast macrophage protein EMP (g3789917)	BLAST
5	265	T262 T7 S97 S155 S191 T261	N2	Transmembrane domain V64-K92 GNS1-SUR4 Integral memb. Protein family E10-E265	Membrane glycoprotein CIG30 (g2289244)	HMMER BLAST PFAM/BLOCKS
6	328	S24 S14 S91 S188		SH3 domain Y254-A308	Colon cancer antigen NY-CO-31 (g3170194)	HMMER BLAST PFAM PRINTS

Table 2 (cont'd)

Poly-peptide Seq ID No:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Genbank homologs	Homologous sequences	Analytical Methods
7	202	S104 T132 S147 S158 S63 S124		Signal peptide M1-A26 Growth factor and cytokine family G108- S114	Vitelline membrane outer layer protein I (g487906)	HMMER BLAST SPscan Motifs
8	96	S82 T91 S96 S98	G46	Transmembrane domain C11-Y27		HMMER
9	651	S404 T68 T110 S132 S179 S206 S222 T265 T508 S571 S575 S587 S601 S634 T19 S85 T97 S178 T228 S264 S310 S363 S419 T425 T452 S527	N29 N176 N503 N569	Transmembrane domain S458-G475	Human sperm specific surface protein (g3116015)	HMMER BLAST
10	443	T105 T89 T98 S224 S240 S242 S254 S365 S366 T127 S330 S427 S435	N96	Signal peptide M1-G33 Transmembrane domain F374-L393 Glucocorticoid receptor G238-I258 Von Willebrand factor type A domain R3111- L328	Lpel10p [Saccharomyces cerevisiae] (g1079682)	SPScan BLIMPS_PRINTS MOTIFS HMMER BLAST_genbank
11	667	S53 T108 T120 S160 T203 T310 T335 S341 S346 S347 T359 T396 T400 T447 S467 T543 S77 S107 S110 S284 T322 T323 T390 T460 T493 S549 S650 Y624 Y665	N184 N282 N326 N640	Signal peptide M1-S35 Transmembrane domain M594-A612 ATP/GTP-binding site motif A (P loop) A85- S92	Neurexin III-beta [Bos taurus] (g451076)	SPScan BLAST_genbank MOTIFS HMMER

Table 2 (cont'd)

Poly-peptide Seq ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Genbank homologs	Homologous sequences	Analytical Methods
12	475	S134 T192 S123 S315 S348 T375 S437 Y124 Y188	N132 N373 N376 N386 N441	Signal peptide M1-P60 Transmembrane domain A157-W177	Erythroid membrane- associated protein ERMAP [Mus musculus] (g6901674)	SPScan BLAST_genbank MOTIFS HMMER
13	479	S50 S62 T295 S42 T45 T386 T452	N48 N284 N442	Signal peptide M1-Y32 Transmembrane domain M300-V322; L398-L416; L151-N171; L16-Y32; N342-A362; Y84-M106 Ammonium transport family I21-Y435 Rhesus blood group protein R10-V28; Y60- L77; V89-M106; N127- L143; Q150-L173; I184-L199; L219-S237; A249-L271; N284-A297; L307-T325; L399-P424	Erythrocyte membrane glycoprotein Rh 50 [Homo sapiens] (g2909819)	SPScan BLIMPS_BLOCKS BLIMPS_PRINTS BLAST_genbank MOTIFS HMMER HMMER_PFAM
14	599	T128 T85 S94 S156 S157 S164 S179 T221 T271 T287 S315 S317 S372 T408 T20 T124 T127 T147 S173 T176 T235 T250 T295 S335 S451 S454 T463 T472	N214 N269 N345 N415		Lamina-associated polypeptide 1C [Rattus norvegicus] (LAP1C) (g769855)	BLAST_genbank MOTIFS
15	299	S52 T218 T232 S149 T197 S227 S283 Y207	N189	Signal peptide M1-T23	Serum lectin P35 [Homo sapiens] (g1369904)	SPScan BLAST_genbank MOTIFS HMMER
16	359	S25 S106 T107 S113 S130 S138 S158 S248 S258 S275 T305 S311 T49 S75 S209 S231			TOP AP integral membrane protein [Gallus gallus] (g642486)	BLAST_genbank MOTIFS

Table 2 (cont'd)

Poly-peptide Seq ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Genbank homologs	Homologous sequences	Analytical Methods
17	160	T98 S119 S125 S148	N78 N130 N146	Signal peptide M1-A53 Transmembrane domain L30-W49	Lectin-like NK cell receptor LLT1 [Homo sapiens] (g6651065)	SPScan BLAST_genbank MOTIFS HMMER

Table 3

Nucleotide SEQ ID NO:	Nucleotide Range of Useful Fragment	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
18	22-67	Hematopoietic/Immune (0.233) Gastrointestinal (0.186) Reproductive (0.163)	Cancer and cell proliferation (0.479) Inflammation (0.396)	PBLUESCRIPT
19	702-747	Hematopoietic/Immune (0.333) Nervous (0.333) Reproductive (0.222)	Cancer and cell proliferation (0.500) Inflammation (0.333)	pINCY
20	649-694	Gastrointestinal (0.611) Reproductive (0.222) Cardiovascular (0.111)	Cancer and cell proliferation (0.579) Inflammation (0.421)	pINCY
21	298-342	Reproductive (0.200) Nervous (0.192) Gastrointestinal (0.125) Hematopoietic/Immune (0.125)	Cancer and cell proliferation (0.684) Inflammation (0.265)	PSPORT1
22	417-462	Reproductive (0.286) Gastrointestinal (0.214) Musculoskeletal (0.143) Nervous (0.143)	Cancer and cell proliferation (0.667) Inflammation (0.133)	pINCY
23	460-505	Reproductive (0.500) Gastrointestinal (0.143) Hematopoietic/Immune (0.143) Nervous (0.143)	Cancer and cell proliferation (0.647) Inflammation (0.294)	pINCY
24	254-299	Developmental (0.200) Hematopoietic/Immune (0.200) Musculoskeletal (0.200) Nervous (0.200)	Cancer and cell proliferation (0.400) Inflammation (0.400)	pINCY
25	120-166	Reproductive (0.258) Hematopoietic/Immune (0.204) Nervous (0.129)	Cancer and cell proliferation (0.636) Inflammation (0.366)	pINCY
26	388-433	Nervous (0.292) Reproductive (0.169) Gastrointestinal (0.135) Hematopoietic/Immune (0.135)	Cancer and cell proliferation (0.629) Inflammation (0.320)	pINCY
27	159-203 1101-1145	Gastrointestinal (0.281) Reproductive (0.219) Nervous (0.188)	Cancer and cell proliferation (0.594) Inflammation (0.344)	PSPORT1



Table 3 (cont'd)

Nucleotide SEQ ID NO:	Nucleotide Range of Useful Fragment	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
28	662-706 1802-1846	Nervous (0.600) Gastrointestinal (0.200) Reproductive (0.133)	Cancer and cell proliferation (0.267) Inflammation (0.467)	pINCY
29	400-444	Developmental (0.176) Gastrointestinal (0.176) Hematopoietic/Immune (0.176) Nervous (0.176)	Cancer and cell proliferation (0.588) Inflammation (0.294)	PSPORT1
30	301-345 1207-1251	Gastrointestinal (0.286) Reproductive (0.286) Urologic (0.214)	Cancer and cell proliferation (0.643) Inflammation (0.357)	PSPORT1
31	461-505 1325-1369	Reproductive (0.319) Hematopoietic/Immune (0.159) Nervous (0.145)	Cancer and cell proliferation (0.565) Inflammation (0.348)	PSPORT1
32	487-531	Cardiovascular (0.667)	Cancer and cell proliferation (0.630) Inflammation (0.370)	pINCY
33	507-551	Reproductive (0.296) Gastrointestinal (0.222) Cardiovascular (0.185)	Cancer and cell proliferation (0.593) Inflammation (0.259)	pINCY
34	163-207	Dermatologic (0.500) Reproductive (0.500)	Cancer and cell proliferation (0.500)	pINCY

Table 4

Nucleotide SEQ ID NO:	Library	Library Comment
18	TMLR3DT01	Library was constructed using RNA isolated from non-adherent and adherent peripheral blood mononuclear cells collected from two unrelated Caucasian male donors (25 and 29 years old). Cells from each donor were purified on Ficoll Hypaque, then co-cultured for 96 hours in medium containing normal human serum at a cell density of $2 \times 10^6$ cells/ml.
19	PGANNOT03	Library was constructed using RNA isolated from paranglionic tumor tissue removed from the intra-abdominal region of a 46-year-old Caucasian male during exploratory laparotomy. Pathology indicated a benign paranglioma and was associated with a grade 2 renal cell carcinoma, clear cell type, which did not penetrate the capsule.
20	COLNNOT23	Library was constructed using RNA isolated from diseased colon tissue removed from a 16-year-old Caucasian male during a total colectomy with abdominal/peritoneal resection. Pathology indicated gastritis and pancolitis consistent with the acute phase of ulcerative colitis. Inflammation was more severe in the transverse colon, with inflammation confined to the mucosa. There was only mild involvement of the ascending and sigmoid colon. Family history included irritable bowel syndrome.
21	BRAITUT02	Library was constructed using RNA isolated from brain tumor tissue removed from the frontal lobe of a 58-year-old Caucasian male during excision of a cerebral meningeal lesion. Pathology indicated a grade 2 metastatic hypernephroma. Patient history included a grade 2 renal cell carcinoma, insomnia, and chronic airway obstruction. Family history included a malignant neoplasm of the kidney.
22	OVARTUT04	Library was constructed using RNA isolated from ovarian tumor tissue removed from a 53-year-old Caucasian female during a total abdominal hysterectomy, removal of the fallopian tubes and ovaries, regional lymph node excision, peritoneal tissue destruction, and incidental appendectomy. Pathology indicated grade 1 transitional cell carcinoma of the right ovary. The left ovary had a hemorrhagic corpus luteum. The uterus had multiple leiomyomas (1 submucosal, 11 intramural), and the endometrium was inactive. The cul-de-sac contained abundant histiocytes and rare clusters of mesothelial cells. Patient history included breast fibrosclerosis and chronic stomach ulcer. Family history included acute stomach ulcer with perforation, breast cancer, bladder cancer, rectal/anal cancer, benign hypertension, coronary angioplasty, and hyperlipidemia.

Table 4 (cont'd)

Nucleotide SEQ ID NO:	Library	Library Comment
23	OVARTUT04	Library was constructed using RNA isolated from ovarian tumor tissue removed from a 53-year-old Caucasian female during a total abdominal hysterectomy, removal of the fallopian tubes and ovaries, regional lymph node excision, peritoneal tissue destruction, and incidental appendectomy. Pathology indicated grade 1 transitional cell carcinoma of the right ovary. The left ovary had a hemorrhagic corpus luteum. The uterus had multiple leiomyomas (1 submucosal, 11 intramural), and the endometrium was inactive. The cul-de-sac contained abundant histiocytes and rare clusters of mesothelial cells. Patient history included breast fibrosclerosis and chronic stomach ulcer. Family history included acute stomach ulcer with perforation, breast cancer, bladder cancer, rectal/anal cancer, benign hypertension, coronary angioplasty, and hyperlipidemia.
24	KIDNNOT25	Library was constructed using RNA isolated from kidney tissue removed from the left lower kidney pole of a 42-year-old Caucasian female during nephroureterectomy. Pathology indicated slight hydronephrosis and nephrolithiasis. Patient history included calculus of the kidney.
25	ENDINOT02	Library was constructed using RNA isolated from treated iliac artery endothelial cells removed from a Black female. The cells were treated with TNF alpha (10ng/ml) and IL-1 beta (10ng/ml) for 20 hours.
26	KIDCTMT01	Library was constructed using RNA isolated from kidney cortex tissue removed from a 65-year-old male during nephroureterectomy. Pathology for the associated tumor tissue indicated grade 3 renal cell carcinoma within the mid-portion of the kidney and the renal capsule.
27	BRSTTUT02	Library was constructed using RNA isolated from breast tumor tissue removed from a 54-year-old Caucasian female during a bilateral radical mastectomy with reconstruction. Pathology indicated residual invasive grade 3 mammary ductal adenocarcinoma. The remaining breast parenchyma exhibited proliferative fibrocystic changes without atypia. One of 10 axillary lymph nodes had metastatic tumor as a microscopic intranodal focus. Patient history included kidney infection and condyloma acuminatum. Family history included benign hypertension, hyperlipidemia, and a malignant colon neoplasm.
28	BRAITUT07	Library was constructed using RNA isolated from left frontal lobe tumor tissue removed from the brain of a 32-year-old Caucasian male during excision of a cerebral meningeal lesion. Pathology indicated low grade desmoplastic neuronal neoplasm, type not otherwise specified. The lesion formed a firm, circumscribed cyst-associated mass involving white matter and cortex. No definite glial component was evident to suggest a diagnosis of ganglioglioma. Family history included atherosclerotic coronary artery disease.
29	LUNGAST01	Library was constructed using RNA isolated from the lung tissue of a 17-year-old Caucasian male, who died from head trauma. Patient history included asthma.

Table 4 (cont'd)

Nucleotide SEQ ID NO:	Library	Library Comment
30	BEPINOT01	Library was constructed using RNA isolated from a bronchial epithelium primary cell line derived from a 54-year-old Caucasian male.
31	PROSNON01	Library was constructed from 4.4 million independent clones from a normal prostate library. Starting RNA was made from prostate tissue removed from a 28-year-old Caucasian male who died from a self-inflicted gunshot wound. The normalization and hybridization conditions were adapted from Soares, M.B. et al. (1994) Proc. Natl. Acad. Sci. USA 91:9228-9232, using a longer (19 hour) reannealing hybridization period.
32	LUNGNOT22	Library was constructed using RNA isolated from lung tissue removed from a 58-year-old Caucasian female. The tissue sample used to construct this library was found to have tumor contaminant upon microscopic examination. Pathology for the associated tumor tissue indicated a caseating granuloma. Family history included congestive heart failure, breast cancer, secondary bone cancer, acute myocardial infarction and atherosclerotic coronary artery disease.
33	OVARNOT09	Library was constructed using RNA isolated from ovarian tissue removed from a 28-year-old Caucasian female during a vaginal hysterectomy and removal of the fallopian tubes and ovaries. Pathology indicated multiple follicular cysts ranging in size from 0.4 to 1.5 cm in the right and left ovaries, chronic cervicitis and squamous metaplasia of the cervix, and endometrium in weakly proliferative phase. Family history included benign hypertension, hyperlipidemia, and atherosclerotic coronary artery disease.
34	FIBRTXT02	Library was constructed using RNA isolated from treated dermal fibroblast tissue removed from the breast of a 31-year-old Caucasian female. The cells were treated with 9CIS Retinoic Acid, 1 microM for 20 hours.

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

5 a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-17,  
b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-17,

c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17, and

10 d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-17.

2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:1-17.

15 3. An isolated polynucleotide encoding a polypeptide of claim 1.

4. An isolated polynucleotide of claim 3 selected from the group consisting of SEQ ID NO:18-34.

20 5. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.

6. A cell transformed with a recombinant polynucleotide of claim 5.

25 7. A transgenic organism comprising a recombinant polynucleotide of claim 5.

8. A method for producing a polypeptide of claim 1, the method comprising:

30 a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and

b) recovering the polypeptide so expressed.

35 9. An isolated antibody which specifically binds to a polypeptide of claim 1.

10. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:18-34,
- b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:18-34,
- c) a polynucleotide sequence complementary to a),
- d) a polynucleotide sequence complementary to b), and
- e) an RNA equivalent of a)-d).

11. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 10.

12. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 10, the method comprising:

- a) hybridizing the sample with a probe comprising at least 16 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and
- b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

13. A method of claim 12, wherein the probe comprises at least 30 contiguous nucleotides.

14. A method of claim 12, wherein the probe comprises at least 60 contiguous nucleotides.

15. A pharmaceutical composition comprising an effective amount of a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

16. A method for treating a disease or condition associated with decreased expression of functional HUMAP, comprising administering to a patient in need of such treatment the pharmaceutical composition of claim 15.

17. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and

b) detecting agonist activity in the sample.

18. A pharmaceutical composition comprising an agonist compound identified by a method of claim 17 and a pharmaceutically acceptable excipient.

5

19. A method for treating a disease or condition associated with decreased expression of functional HUMAP, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 18.

10 20. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting antagonist activity in the sample.

15 21. A pharmaceutical composition comprising an antagonist compound identified by a method of claim 20 and a pharmaceutically acceptable excipient.

20 22. A method for treating a disease or condition associated with overexpression of functional HUMAP, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 21.

23. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 4, the method comprising:

- 25
- a) exposing a sample comprising the target polynucleotide to a compound, and
  - b) detecting altered expression of the target polynucleotide.

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(54) Title: HUMAN MEMBRANE-ASSOCIATED PROTEINS

(57) Abstract: The invention provides human membrane-associated proteins (HUMAP) and polynucleotides which identify and encode HUMAP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HUMAP.

WO 00/65054 A3



## SEQUENCE LISTING

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WO 00/65054

PCT/US00/10884

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WO 00/65054

PCT/US00/10884

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WO 00/65054

PCT/US00/10884

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Leu	Glu	Pro	Val	Asp	Phe	Leu	Gly	Lys	Ala	Lys	Val	Val	Ala	Ser
				50					55					60
Ala	Ile	Pro	Asp	Asp	Gln	Gly	Trp	Gly	Val	Arg	Pro	Gln	Gln	Pro
				65					70					75
Gln	Gly	Pro	Gly	Ala	Asn	His	Asp	Ala	Arg	Ser	Leu	Ile	Met	Asp
				80					85					90
Ser	Pro	Arg	Ala	Gly	Thr	His	Gln	Gly	Pro	Leu	Asp	Ala	Glu	Thr
				95					100					105
Glu	Val	Gly	Ala	Asp	Arg	Cys	Thr	Ser	Thr	Ala	Tyr	Gln	Glu	Gln
				110					115					120
Arg	Pro	Gln	Val	Glu	Gln	Val	Gly	Lys	Gln	Ala	Pro	Leu	Ser	Pro
				125					130					135
Gly	Leu	Pro	Ala	Met	Gly	Gly	Pro	Gly	Pro	Gly	Pro	Cys	Glu	Asp
				140					145					150
Pro	Ala	Gly	Ala	Gly	Gly	Val	Gly	Ala	Gly	Gly	Ser	Glu	Pro	Leu
				155					160					165
Val	Thr	Val	Thr	Val	Gln	Cys	Ala	Phe	Thr	Val	Ala	Leu	Arg	Ala
				170					175					180
Arg	Arg	Gly	Ala	Asp	Leu	Ser	Ser	Leu	Arg	Ala	Leu	Leu	Gly	Gln

WO 00/65054

PCT/US00/10884

Ala	Leu	Pro	His	185	Gln	Ala	Gln	Leu	Gly	190	Gln	Leu	Ser	Tyr	Leu	Ala	195
Pro	Gly	Glu	Asp	200	Gly	His	Trp	Val	Pro	205	Ile	Pro	Glu	Glu	Glu	Ser	210
Leu	Gln	Arg	Ala	215	Trp	Gln	Asp	Ala	Ala	220	Ala	Cys	Pro	Arg	Gly	Leu	225
Gln	Leu	Gln	Cys	230	Arg	Gly	Ala	Gly	Gly	235	Arg	Pro	Val	Leu	Tyr	Gln	240
Val	Val	Ala	Gln	245	His	Ser	Tyr	Ser	Ala	250	Gln	Gly	Pro	Glu	Asp	Leu	255
Gly	Phe	Arg	Gln	260	Gly	Asp	Thr	Val	Asp	265	Val	Leu	Cys	Glu	Val	Asp	270
Gln	Ala	Trp	Leu	275	Glu	Gly	His	Cys	Asp	280	Gly	Arg	Ile	Gly	Ile	Phe	285
Pro	Lys	Cys	Phe	290	Val	Val	Pro	Ala	Gly	295	Pro	Arg	Met	Ser	Gly	Ala	300
Pro	Gly	Arg	Leu	305	Pro	Arg	Ser	Gln	Gln	310	Gly	Asp	Gln	Pro			315
				320						325							

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 <223> Incyte ID No.: 3536577CD1

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Leu	Arg	Ala	Thr	Gly	Phe	Thr	Cys	Ala	Gln	Ala	Asp	Gly	Arg	Asn			30
				20				25									35
Gly	Tyr	Thr	Ala	Val	Ile	Glu	Val	Thr	Ser	Gly	Gly	Pro	Trp	Gly			45
				35				40									50
Asp	Trp	Ala	Trp	Pro	Glu	Met	Cys	Pro	Asp	Gly	Phe	Phe	Ala	Ser			60
				50				55									65
Gly	Phe	Ser	Leu	Lys	Val	Glu	Pro	Pro	Gln	Gly	Ile	Pro	Gly	Asp			75
				65				70									80
Asp	Thr	Ala	Leu	Asn	Gly	Ile	Arg	Leu	His	Cys	Ala	Arg	Gly	Asn			90
				80				85									95
Val	Leu	Gly	Asn	Thr	His	Val	Val	Glu	Ser	Gln	Ser	Gly	Ser	Trp			105
				95				100									110
Gly	Glu	Trp	Ser	Glu	Pro	Leu	Trp	Cys	Arg	Gly	Gly	Ala	Tyr	Leu			120
				110				115									125
Val	Ala	Phe	Ser	Leu	Arg	Val	Glu	Ala	Pro	Thr	Thr	Leu	Gly	Asp			135
				125				130									140
Asn	Thr	Ala	Ala	Asn	Asn	Val	Arg	Phe	Arg	Cys	Ser	Asp	Gly	Glu			150
				140				145									155
Glu	Leu	Gln	Gly	Pro	Gly	Leu	Ser	Trp	Gly	Asp	Phe	Gly	Asp	Trp			165
				155				160									170
Ser	Asp	His	Cys	Pro	Lys	Gly	Ala	Cys	Gly	Leu	Gln	Thr	Lys	Ile			180
				170				175									185
Gln	Gly	Pro	Arg	Gly	Leu	Gly	Asp	Asp	Thr	Ala	Leu	Asn	Asp	Ala			195
				185				190									
Arg	Leu	Phe	Cys	Cys	Arg	Ser											200

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<220>



WO 00/65054

PCT/US00/10884

	275		280		285
Met Trp Leu Gly	Ala Gln Asn Gly Trp	Leu Tyr Val His Ser	Ala		
	290		295		300
Val Ala Asn Trp	Lys Lys Cys Leu His	Ser Ile Lys Leu Lys	Asp		
	305		310		315
Ser Val Leu Ser	Leu Val His Val Lys	Gly Arg Val Leu Val	Ala		
	320		325		330
Leu Ala Asp Gly	Thr Leu Ala Ile Phe	His Arg Gly Glu Asp	Gly		
	335		340		345
Gln Trp Asp Leu	Ser Asn Tyr His Leu	Met Asp Leu Gly His	Pro		
	350		355		360
His His Ser Ile	Arg Cys Met Ala Val	Val Tyr Asp Arg Val	Trp		
	365		370		375
Cys Gly Tyr Lys	Asn Lys Val His Val	Ile Gln Pro Lys Thr	Met		
	380		385		390
Gln Ile Glu Lys	Ser Phe Asp Ala His	Pro Arg Arg Glu Ser	Gln		
	395		400		405
Val Arg Gln Leu	Ala Trp Ile Gly Asp	Gly Val Trp Val Ser	Ile		
	410		415		420
Arg Leu Asp Ser	Thr Leu Arg Leu Tyr	His Ala His Thr His	Gln		
	425		430		435
His Leu Gln Asp	Val Asp Ile Glu Pro	Tyr Val Ser Lys Met	Leu		
	440		445		450
Gly Thr Gly Lys	Leu Gly Phe Ser Phe	Val Arg Ile Thr Ala	Leu		
	455		460		465
Leu Val Ala Gly	Ser Arg Leu Trp Val	Gly Thr Gly Asn Gly	Val		
	470		475		480
Val Ile Ser Ile	Pro Leu Thr Glu Thr	Val Val Leu His Arg	Gly		
	485		490		495
Gln Leu Leu Gly	Leu Arg Ala Asn Lys	Thr Ser Pro Thr Ser	Gly		
	500		505		510
Glu Gly Ala Arg	Pro Gly Gly Ile Ile	His Val Tyr Gly Asp	Asp		
	515		520		525
Ser Ser Asp Arg	Ala Ala Ser Ser Phe	Ile Pro Tyr Cys Ser	Met		
	530		535		540
Ala Gln Ala Gln	Leu Cys Phe His Gly	His Arg Asp Ala Val	Lys		
	545		550		555
Phe Phe Val Ser	Val Pro Gly Asn Val	Leu Ala Thr Leu Asn	Gly		
	560		565		570
Ser Val Leu Asp	Ser Pro Ala Glu Gly	Pro Gly Pro Ala Ala	Pro		
	575		580		585
Ala Ser Glu Val	Glu Gly Gln Lys Leu	Arg Asn Val Leu Val	Leu		
	590		595		600
Ser Gly Gly Glu	Gly Tyr Ile Asp Phe	Arg Ile Gly Asp Gly	Glu		
	605		610		615
Asp Asp Glu Thr	Glu Glu Gly Ala Gly	Asp Met Ser Gln Val	Lys		
	620		625		630
Pro Val Leu Ser	Lys Ala Glu Arg Ser	His Ile Ile Val Trp	Gln		
	635		640		645
Val Ser Tyr Thr	Pro Glu				
	650				

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&lt;211&gt; 443

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No.: 645566CD1

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Met Glu Cys Leu Arg	Ser Leu Pro Cys Leu Leu Pro Arg Ala Met		
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Arg Leu Pro Arg Arg	Thr Leu Cys Ala Leu Ala Leu Asp Val Thr		
	20	25	30





WO 00/65054

PCT/US00/10884

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Met His Leu Arg Ile His Ala Arg Arg Ser Pro Pro Arg Arg Pro
 1      5      10      15
Ala Trp Thr Leu Gly Ile Trp Phe Leu Phe Trp Gly Cys Ile Val
 20      25      30
Ser Ser Val Trp Ser Ser Ser Asn Val Ala Ser Ser Ser Ser Thr
 35      40      45
Ser Ser Ser Pro Gly Ser His Ser Gln His Glu His His Phe His
 50      55      60
Gly Ser Lys His His Ser Val Pro Ile Ser Ile Tyr Arg Ser Pro
 65      70      75
Val Ser Leu Arg Gly Gly His Ala Gly Ala Thr Tyr Ile Phe Gly
 80      85      90
Lys Ser Gly Gly Leu Ile Leu Tyr Thr Trp Pro Ala Asn Asp Arg
 95      100      105
Pro Ser Thr Arg Ser Asp Arg Leu Ala Val Gly Phe Ser Thr Thr
 110      115      120
Val Lys Asp Gly Ile Leu Val Arg Ile Asp Ser Ala Pro Gly Leu
 125      130      135
Gly Asp Phe Leu Gln Leu His Ile Glu Gln Gly Lys Ile Gly Val
 140      145      150
Val Phe Asn Ile Gly Thr Val Asp Ile Ser Ile Lys Glu Glu Arg
 155      160      165
Thr Pro Val Asn Asp Gly Lys Tyr His Val Val Arg Phe Thr Arg
 170      175      180
Asn Gly Gly Asn Ala Thr Leu Gln Val Asp Asn Trp Pro Val Asn
 185      190      195
Glu His Tyr Pro Thr Gly Asn Thr Asp Asn Glu Arg Phe Gln Met
 200      205      210
Val Lys Gln Lys Ile Pro Phe Lys Tyr Asn Arg Pro Val Glu Glu
 215      220      225
Trp Leu Gln Glu Lys Gly Arg Gln Leu Thr Ile Phe Asn Thr Gln
 230      235      240
Ala Gln Ile Ala Ile Gly Gly Lys Asp Lys Gly Arg Leu Phe Gln
 245      250      255
Gly Gln Leu Ser Gly Leu Tyr Tyr Asp Gly Leu Lys Val Leu Asn
 260      265      270
Met Ala Ala Glu Asn Asn Pro Asn Ile Lys Ile Asn Gly Ser Val
 275      280      285
Arg Leu Val Gly Glu Val Pro Ser Ile Leu Gly Thr Thr Gln Thr
 290      295      300
Thr Ser Met Pro Pro Glu Met Ser Thr Thr Val Met Glu Thr Thr
 305      310      315
Thr Thr Met Ala Thr Thr Thr Thr Arg Lys Asn Arg Ser Thr Ala
 320      325      330
Ser Ile Gln Pro Thr Ser Asp Asp Leu Val Ser Ser Ala Glu Cys
 335      340      345
Ser Ser Asp Asp Glu Asp Phe Val Glu Cys Glu Pro Ser Thr Gly
 350      355      360
Gly Glu Leu Val Ile Pro Leu Leu Val Glu Asp Pro Leu Ala Thr
 365      370      375
Pro Pro Ile Ala Thr Arg Ala Pro Ser Ile Thr Leu Pro Pro Thr
 380      385      390
Phe Arg Pro Leu Leu Thr Ile Ile Glu Thr Thr Lys Asp Ser Leu
 395      400      405
Ser Met Thr Ser Glu Ala Gly Leu Pro Cys Leu Ser Asp Gln Gly
 410      415      420
Ser Asp Gly Cys Asp Asp Asp Gly Leu Val Ile Ser Gly Tyr Gly
 425      430      435
Ser Gly Glu Thr Phe Asp Ser Asn Leu Pro Pro Thr Asp Asp Glu
 440      445      450
Asp Phe Tyr Thr Thr Phe Ser Leu Val Thr Asp Lys Ser Leu Ser
 455      460      465
Thr Ser Ile Phe Glu Gly Gly Tyr Lys Ala His Ala Pro Lys Trp
 470      475      480
Glu Ser Lys Asp Phe Arg Pro Asn Lys Val Ser Glu Thr Ser Arg
 485      490      495

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WO 00/65054

PCT/US00/10884

Thr	Thr	Thr	Thr	Ser	Leu	Ser	Pro	Glu	Leu	Ile	Arg	Phe	Thr	Ala	
				500					505					510	
Ser	Ser	Ser	Ser	Gly	Met	Val	Pro	Lys	Leu	Pro	Ala	Gly	Lys	Met	
				515					520					525	
Asn	Asn	Arg	Asp	Leu	Lys	Pro	Gln	Pro	Asp	Ile	Val	Leu	Leu	Pro	
				530					535					540	
Leu	Pro	Thr	Ala	Tyr	Glu	Leu	Asp	Ser	Thr	Lys	Leu	Lys	Ser	Pro	
				545					550					555	
Leu	Ile	Thr	Ser	Pro	Met	Phe	Arg	Asn	Val	Pro	Thr	Ala	Asn	Pro	
				560					565					570	
Thr	Glu	Pro	Gly	Ile	Arg	Arg	Val	Pro	Gly	Ala	Ser	Glu	Val	Ile	
				575					580					585	
Arg	Glu	Ser	Ser	Ser	Thr	Thr	Gly	Met	Val	Val	Gly	Ile	Val	Ala	
				590					595					600	
Ala	Ala	Ala	Leu	Cys	Ile	Leu	Ile	Leu	Leu	Tyr	Ala	Met	Tyr	Lys	
				605					610					615	
Tyr	Arg	Asn	Arg	Asp	Glu	Gly	Ser	Tyr	Gln	Val	Asp	Glu	Thr	Arg	
				620					625					630	
Asn	Tyr	Ile	Ser	Asn	Ser	Ala	Gln	Ser	Asn	Gly	Thr	Leu	Met	Lys	
				635					640					645	
Glu	Lys	Gln	Gln	Ser	Ser	Lys	Ser	Gly	His	Lys	Lys	Gln	Lys	Asn	
				650					655					660	
Lys	Asp	Arg	Glu	Tyr	Tyr	Val									
				665											

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Ile	Pro	Leu	Val	Phe	Leu	Arg	Leu	Ser	Val	Tyr	Val	Ser	Gly	His	
				20					25					30	
Ala	Gly	Asp	Ala	Gly	Lys	Phe	His	Val	Ala	Leu	Leu	Gly	Gly	Thr	
				35					40					45	
Ala	Glu	Leu	Leu	Cys	Pro	Leu	Ser	Leu	Trp	Pro	Gly	Thr	Val	Pro	
				50					55					60	
Lys	Glu	Val	Arg	Trp	Leu	Arg	Ser	Pro	Phe	Pro	Gln	Arg	Ser	Gln	
				65					70					75	
Ala	Val	His	Ile	Phe	Arg	Asp	Gly	Lys	Asp	Gln	Asp	Glu	Asp	Leu	
				80					85					90	
Met	Pro	Glu	Tyr	Lys	Gly	Arg	Thr	Val	Leu	Val	Arg	Asp	Ala	Gln	
				95					100					105	
Glu	Gly	Ser	Val	Thr	Leu	Gln	Ile	Leu	Asp	Val	Arg	Leu	Glu	Asp	
				110					115					120	
Gln	Gly	Ser	Tyr	Arg	Cys	Leu	Ile	Gln	Val	Gly	Asn	Leu	Ser	Lys	
				125					130					135	
Glu	Asp	Thr	Val	Ile	Leu	Gln	Val	Ala	Ala	Pro	Ser	Val	Gly	Ser	
				140					145					150	
Leu	Ser	Pro	Ser	Ala	Val	Ala	Leu	Ala	Val	Ile	Leu	Pro	Val	Leu	
				155					160					165	
Val	Leu	Leu	Ile	Met	Val	Cys	Leu	Cys	Leu	Ile	Trp	Lys	Gln	Arg	
				170					175					180	
Arg	Ala	Lys	Glu	Lys	Leu	Leu	Tyr	Glu	His	Val	Thr	Glu	Val	Asp	
				185					190					195	
Asn	Leu	Leu	Ser	Asp	His	Ala	Lys	Glu	Lys	Gly	Lys	Leu	His	Lys	
				200					205					210	
Ala	Val	Lys	Lys	Leu	Arg	Ser	Glu	Leu	Lys	Leu	Lys	Arg	Ala	Ala	
				215					220					225	
Ala	Asn	Ser	Gly	Trp	Arg	Arg	Ala	Arg	Leu	His	Phe	Val	Ala	Val	



WO 00/65054

PCT/US00/10884

Glu Phe Ile Leu	155	Val Lys Asp Ala Gly	165
Leu Asn Leu Leu Lys	160	Gly Gly	165
Ser Met Thr Ile	170	Phe Gly Leu Thr	180
His Thr Phe Gly Ala	175	Val	180
Thr Arg Ile Leu	185	Glu Gln Ser Lys Glu	195
Tyr Arg Arg Asn Leu	190	Arg	195
Gln Asn Ser Val	200	Phe Ala Met Ile Gly	210
Tyr Gln Ser Asp Leu	205	Thr	210
Leu Phe Leu Trp	215	Asn Ser Ala Ile	225
Met Tyr Trp Pro Ser	220	Ser	225
Tyr His Gly Asp	230	Ile Asn Thr Tyr	240
Ser Gln His Arg Ala	235	Cys	240
Ser Leu Ala Ala	245	Val Ala Ile Ser	255
Cys Val Leu Thr Ser	250	Ala	255
Leu His Lys Lys	260	Val His Ile Gln Asn	270
Gly Lys Leu Asp Met	265	Ala	270
Thr Leu Ala Gly	275	Thr Ala Ala Glu Met	285
Gly Val Ala Val Gly	280	Met	285
Leu Met Pro Tyr	290	Gly Phe Val Cys Gly	300
Gly Ala Leu Ile Ile	295	Ile	300
Ile Ser Thr Leu	305	Thr Pro Phe Leu Glu	315
Gly Phe Val Tyr Leu	310	Ser	315
Arg Leu His Ile	320	Ile Asn Asn Leu His	330
Gln Asp Thr Cys Gly	325	Gly	330
Ile Pro Gly Ile	335	Ala Val Thr Ala	345
Ile Gly Gly Ile Val	340	Ala	345
Ser Ala Ser Leu	350	Gly Leu Val His	360
Glu Val Tyr Gly Lys	355	Ser	360
Phe Asp Phe Gln	365	Thr Ala Arg Thr	375
Gly Phe Asn Gly Asp	370	Gln	375
Gly Lys Phe Gln	380	Val Thr Leu Ala Met	390
Ile Tyr Gly Leu Leu	385	Ala	390
Leu Met Gly Gly	395	Ile Leu Arg Leu Pro	405
Ile Ile Val Gly Leu	400	Phe	405
Trp Gly Gln Pro	410	Glu Asp Ala Val	420
Ser Asp Glu Asn Cys	415	Tyr	420
Trp Glu Met Pro	425	Val Tyr Ile Pro Glu	435
Gly Asn Ser Thr	430	Asp	435
Pro Thr Phe Lys	440	Val Pro Ser Val Pro	450
Pro Ser Gly Pro Ser	445	Met	450
Val Ser Pro Leu	455	Val Pro Leu Val Pro	465
Pro Met Ala Ser Ser	460		465
	470		475

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Arg Leu Ala Pro	10	Ile Arg Glu Gly Arg	30
Gln Asn Gly Gly Cys	20	Ser Asp Ala Pro Ala	30
Arg Thr Pro Pro	25	Arg Glu Val Arg Phe	45
Ser His Gln Gly Arg	35	Ser	45
Asp Glu Pro Pro	40	Glu Val Arg Phe	60
Glu Val Tyr Gly Asp	50	Val	60
Lys Glu Arg Ser	55	Phe Glu Pro Leu Val	75
Pro Val Gly Lys Arg	65	Ala	75
	70	Thr Arg Leu Glu Glu	90
	80	Phe	90
	85		90



590

595

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Gly	Gly	Pro	Ala	Cys	Leu	Lys	Thr	Gln	Glu	His	Pro	Ser	Cys	Pro
				20					25					30
Gly	Pro	Arg	Glu	Leu	Glu	Ala	Ser	Lys	Val	Val	Leu	Leu	Pro	Ser
				35					40					45
Cys	Pro	Gly	Ala	Pro	Gly	Ser	Pro	Gly	Glu	Lys	Gly	Ala	Pro	Gly
				50					55					60
Pro	Gln	Gly	Pro	Pro	Gly	Pro	Pro	Gly	Lys	Met	Gly	Pro	Lys	Gly
				65					70					75
Glu	Pro	Gly	Asp	Pro	Val	Asn	Leu	Leu	Arg	Cys	Gln	Glu	Gly	Pro
				80					85					90
Arg	Asn	Cys	Arg	Glu	Leu	Leu	Ser	Gln	Gly	Ala	Thr	Leu	Ser	Gly
				95					100					105
Trp	Tyr	His	Leu	Cys	Leu	Pro	Glu	Gly	Arg	Ala	Leu	Pro	Val	Phe
				110					115					120
Cys	Asp	Met	Asp	Thr	Glu	Gly	Gly	Gly	Trp	Leu	Val	Phe	Gln	Arg
				125					130					135
Arg	Gln	Asp	Gly	Ser	Val	Asp	Phe	Phe	Arg	Ser	Trp	Ser	Ser	Tyr
				140					145					150
Arg	Ala	Gly	Phe	Gly	Asn	Gln	Glu	Ser	Glu	Phe	Trp	Leu	Gly	Asn
				155					160					165
Glu	Asn	Leu	His	Gln	Leu	Thr	Leu	Gln	Gly	Asn	Trp	Glu	Leu	Arg
				170					175					180
Val	Glu	Leu	Glu	Asp	Phe	Asn	Gly	Asn	Arg	Thr	Phe	Ala	His	Tyr
				185					190					195
Ala	Thr	Phe	Arg	Leu	Leu	Gly	Glu	Val	Asp	His	Tyr	Gln	Leu	Ala
				200					205					210
Leu	Gly	Lys	Phe	Ser	Glu	Gly	Thr	Ala	Gly	Asp	Ser	Leu	Ser	Leu
				215					220					225
His	Ser	Gly	Arg	Pro	Phe	Thr	Thr	Tyr	Asp	Ala	Asp	His	Asp	Ser
				230					235					240
Ser	Asn	Ser	Asn	Cys	Ala	Val	Ile	Val	His	Gly	Ala	Trp	Trp	Tyr
				245					250					255
Ala	Ser	Cys	Tyr	Arg	Ser	Asn	Leu	Asn	Gly	Arg	Tyr	Ala	Val	Ser
				260					265					270
Glu	Ala	Ala	Ala	His	Lys	Tyr	Gly	Ile	Asp	Trp	Ala	Ser	Gly	Arg
				275					280					285
Gly	Val	Gly	His	Pro	Tyr	Arg	Arg	Val	Arg	Met	Met	Leu	Arg	
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<212> PRT
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WO 00/65054

PCT/US00/10884

Asp	Trp	Leu	Gly	Val	Arg	Asp	Lys	Cys	Phe	Tyr	Phe	Ser	Asp	Asp
				65					70					75
Thr	Arg	Asn	Trp	Thr	Ala	Ser	Lys	Ile	Phe	Cys	Ser	Leu	Gln	Lys
				80					85					90
Ala	Glu	Leu	Ala	Gln	Ile	Asp	Thr	Gln	Glu	Asp	Met	Glu	Phe	Leu
				95					100					105
Lys	Arg	Tyr	Ala	Gly	Thr	Asp	Met	His	Trp	Ile	Gly	Leu	Ser	Arg
				110					115					120
Lys	Gln	Gly	Asp	Ser	Trp	Lys	Trp	Thr	Asn	Gly	Thr	Thr	Phe	Asn
				125					130					135
Gly	Trp	Pro	Ser	Asn	Ser	Lys	Trp	Ser	Cys	Asn	Trp	Ser	Leu	Arg
				140					145					150
Gln	Trp	Leu	Leu	Leu	Leu	Gly	Pro	Leu	Arg					
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 ctgctggacc tcagctaccc ccgcaccac ccggccctgc tgaaagtggc gcaaagtgtc 180  
 accctgctga ttgccttcac ctgtgtgctg agctccctgt ggaccaacta cagcgctac 240  
 agctactttg aagtggtcac catttgcgac ttgataatga tctctgcctt ttacctgtgc 300  
 cacctcttcc gcttctaccg cgtgtctacc tgtatcagct ggcccctgtc ggaacttctg 360  
 cactatttaa tcgggtaccg gctcctctc atcgctcca ttgtggcagc ttccaagagt 420  
 tacaaccaga cgggactggg agccggagcg atctttgggt tcatggccac ctctctctgc 480  
 atggcaagca tatggctgtc ctataagatc tcgtgtgtaa cccagtccac agatgcagcc 540  
 gtctgatgag gccacaaccc ctaggccctc caggagcttt gcagagagga ggacgtgtac 600  
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WO 00/65054

PCT/US00/10884

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WO 00/65054

PCT/US00/10884

<212> DNA  
<213> Homo sapiens

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WO 00/65054

PCT/US00/10884

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WO 00/65054

PCT/US00/10884

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**DECLARATION AND POWER OF ATTORNEY FOR  
UNITED STATES PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

**HUMAN MEMBRANE-ASSOCIATED PROTEINS**

the specification of which:

/ X / is attached hereto.

/ / was filed on \_\_\_\_\_ as application Serial No. \_\_\_\_\_ and if this box contains an X / /, was amended on \_\_\_\_\_.

/ X / was filed as Patent Cooperation Treaty international application No. PCT/US00/10884 on April 20, 2000, if this box contains an X / /, was amended on under Patent Cooperation Treaty Article 19 on \_\_\_\_\_ 2001, and if this box contains an X / /, was amended on \_\_\_\_\_.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim the benefit under Title 35, United States Code, §119 or §365(a)-(b) of any foreign application(s) for patent or inventor's certificate indicated below and of any Patent Cooperation Treaty international applications(s) designating at least one country other than the United States indicated below and have also identified below any foreign application(s) for patent or inventor's certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application for said subject matter the priority of which is claimed:

**Docket No.: PF-0698 USN**

Country	Number	Filing Date	Priority Claimed
_____	_____	_____	// Yes // No
_____	_____	_____	// Yes // No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)
<u>60/130,694</u>	<u>April 23, 1999</u>	<u>Expired</u>
<u>60/140,580</u>	<u>June 23, 1999</u>	<u>Expired</u>

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of Title 35, United States Code §112, I acknowledge my duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)
_____	_____	_____

I hereby appoint the following:

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Michael C. Cerrone	Reg. No. <u>39,132</u>
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Richard C. Ekstrom	Reg. No. <u>37,027</u>
Barrie D. Greene	Reg. No. <u>46,740</u>
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David G. Streeter	Reg. No. <u>43,168</u>
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respectively and individually, as my patent attorneys and/or agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Please address all communications to:

**Docket No.: PF-0698 USN**

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Docket No.: PF-0698 USN

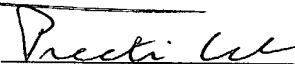
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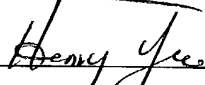
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**Docket No.: PF-0698 USN**

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*700*

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*800*

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